# MACHEREY-NAGEL – NucleoSnap® cfDNA Plasma-Kit (REF 740300.50) for Isolation of S-Monovette® cfDNA Exact stabilized samples

## INTRODUCTION

The biomarker cell free DNA (cfDNA) is playing an increasingly important role in research and diagnostics. For blood sample stabilization SARSTEDT offers the innovative S-Monovette® cfDNA Exact ensuring excellent sample quality with a guaranteed stabilization performance for 14 days at 4 - 37°C (Schrage *et al.* 2022). Compatibility of blood collection tubes (containing cfDNA stabilizer solution) with isolation kits for cfDNA can be impaired due to the fact that cfDNA in the sample can be modified due to fixation by the stabiliser solution prefilled in the tubes. Therefore, we are pleased to show that S-Monovette® cfDNA Exact is compatible with a wide range of cfDNA isolation kits, in particular with NucleoSnap® cfDNA Plasma-Kit from MACHEREY-NAGEL (REF 740300.50) as shown in the following application note. The details, and protocol optimisations, if necessary, are listed below.

## MATERIAL & METHODS

### Blood sample collection and storage:

Blood from four healthy donors was drawn into S-Monovette® cfDNA Exact blood collection tubes (REF 01.2040.001, Sarstedt AG & Co., Nümbrecht, Germany) or S-Monovette® K3 EDTA (REF 01.1605.001, Sarstedt AG & Co., Nümbrecht, Germany).
Plasma was separated within one hour after blood collection (D0) or after storage of the blood collection tube for 3 days at 25°C (D3) by two step centrifugation as described in the cfDNA Exact Monovette´s manual or the protocol below. The plasma was stored at -80°C until cfDNA isolation.

### cfDNA isolation:

1ml of plasma from S-Monovette® cfDNA Exact and S-Monovette® K3 EDTA each at different time points were lysed and cfDNA was isolated as described in the manufacturers manual taking into account the input dependent scaling of the buffer volumes. A detailed protocol can be found below.

### cfDNA analysis:

To assess the cfDNA quality 1 µl of the eluate was visualised electrophoretically on a High Sensitivity DNA Chip (Agilent REF 5067-4626) with the Bioanalyzer 2100. The applicability of the isolated cfDNA to be used in common analysis methods was shown by using the samples in a qPCR assay to amplify the single copy genes *myostatin* (*MSTN;* (Breitbach *et al.* 2014) and a human *endogenous retrovirus* (*ERV-3;* Devonshire *et al.* 2014). The qPCR reactions were run with 8 µl sample input using the Maxima SYBR Green/ROX qPCR Master Mix (REF K0223, Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to manufacturer's protocol on a Mastercycler ep realplex 4S (Eppendorf, Hamburg, Germany) or Real-time Thermal cycler qTOWER$^3$ (Analytic Jena GmbH, Jena, Germany). Primers (see Table 1) were used in a final concentration of 0.5 µM.

Table 1. Primer sequences

| Primer | Sequence | Annealing temp. | Fragment length |
|---|---|---|---|
| ERV-3fw (Devonshire *et al.* 2014) | 5‘-CATGGGAAGCAAGGGAACTAATG-3‘ | 60°C | 135 bp |
| ERV-3rev | 5‘-CCCAGCGAGCAATACAGAATTT-3‘ | | |
| MSTNfw (Breitbach *et al.* 2014) | 5‘-TTGGCTCAAACAACCTGAATCC-3‘ | 60°C | 88 bp |
| MSTNrev | 5‘-TCCTGGGAAGGTTACAGCAAG-3‘ | | |

## RESULTS & DISCUSSION

Following the cfDNA isolation, quality control was carried out via an Bioanalyzer (Agilent) with the High Sensitive DNA Analysis Kit. Figure 1 shows the capillary electrophoretic separations of two donors. The stabilization performance of the S-Monovette® cfDNA Exact becomes particularly clear here, since in contrast to storage of the S-Monovette® K3 EDTA, no release of genomic DNA can be detected on day 3. For the donors shown, the cfDNA peak has a low level, but this is not surprising as the donors are a healthy control group.

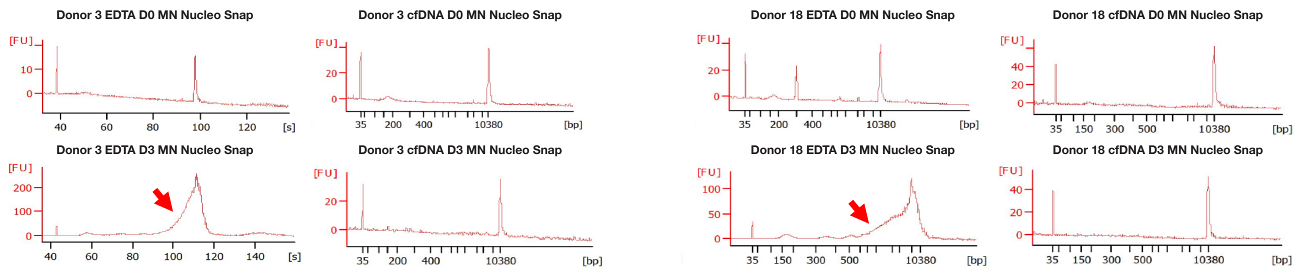


Figure 1: Capillary electrophoretic representation of cfDNA using a Bioanalyzer (Agilent). Illustration of two donors at the time point day 0 and after 3 days at 25°C. The red arrow shows entry of genomic DNA on day 3 into the sample from the S-Monovette® K3 EDTA (not stabilized samples); which is absent in the S-Monovette® cfDNA Exact due to stabilization.

Subsequently, real-time PCRs of the isolated samples from 4 donors with two single-copy genes were performed. The $C_T$ values of the 4 time points are shown in figure 2a. Figure 2b shows the $\Delta C_T$ values calculated to the $C_T$ value from the day 0 samples of the S-Monovette® K3 EDTA which was processed immediately after blood collection (gold standard). Here, both the good isolation efficiency from S-Monovette® cfDNA Exact stabilized samples and the stabilization performance on day 3 at 25°C become clear. No deviations beyond a donor-dependent variation could be detected.

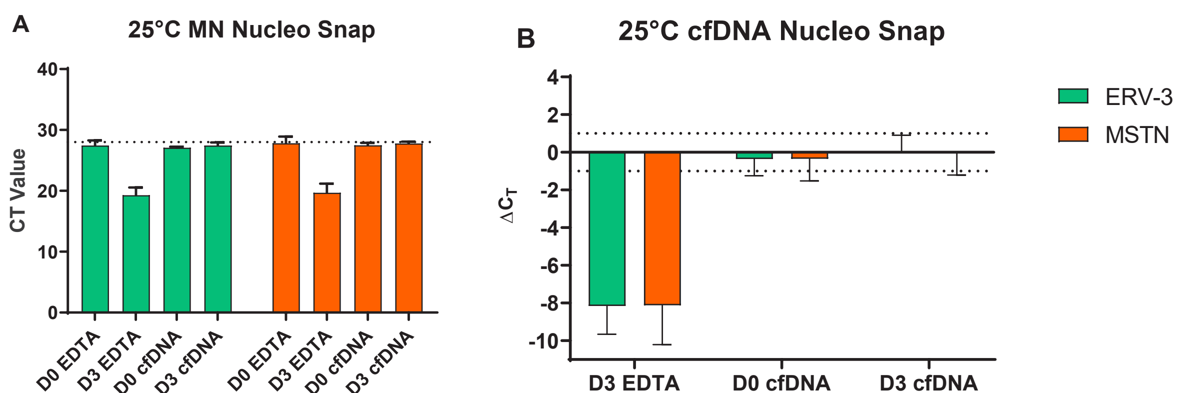


Figure 2: (A) $C_T$ values from the tested single copy genes are shown at different time points. Average values from 4 donors with standard deviations were calculated. (B) Delta $C_T$ value calculation for comparison to EDTA at time 0 were carried out. Values are shown averaged from 4 donors for two single copy genes with standard deviations.

## SUMMARY

In this application note we show the compatibility of S-Monovette® cfDNA Exact stabilized samples with NucleoSnap® cfDNA Plasma-Kit from MACHEREY-NAGEL. With the kit, high quality cfDNA could be isolated from stabilised plasma following the manufacturer's instructions. The Kit is suitable to process up to the maximum plasma yield which can be obtained out of the S-Monovette® cfDNA Exact following the scale up table in the kit´s manual. Additionally, it is shown that sample stabilization of cfDNA samples is indispensable to achieve good sample quality.

## PROTOCOL

Prepare cell-free plasma samples from S-Monovette® cfDNA Exact

1. **Replace the red screw-on lid of S-Monovette® cfDNA Exact with the centrifugation cap included**
2. Centrifuge the blood samples at 2.000-3.000 x g for 10 minutes at room temperature
3. Transfer the plasma into a new centrifugation tube
4. Centrifuge the plasma samples at 15.000 x g for 15 minutes at room temperature and transfer supernatant into a new tube. Continue to isolate cfDNA or store the plasma at -80°C until further use.

Scaling table for sample preparation

| Plasma volume [µl] | Proteinase K [µl] | Buffer VL [µl] | Ethanol [µl] |
|---|---|---|---|
| 1.000 | 15 | 1.000 | 1.000 |
| 2.000 | 30 | 2.000 | 2.000 |
| 3.000 | 45 | 3.000 | 3.000 |
| 4.000 | 60 | 4.000 | 4.000 |
| 5.000 | 75 | 5.000 | 5.000 |

Volumes of wash buffers have not to be scaled, elution can occur in a volume 50 µl elution buffer independent of the sample input volume

Lyse plasma samples

1. Submit **1.000 µl Plasma** to a lysis tube with suitable volume (e.g. 5 ml reaction tube Sarstedt REF. 72.701.400)
2. Add 15 µl Proteinase K, mix
3. Incubate 5 min at room temperature
4. Add **1.000 Buffer VL**, mix thoroughly
5. Place tube(s) into a suitable Thermo Mixer heated up to 56°C with tubes placed inside, and incubate for 10 minutes while mixing (1.000 rpm)
6. Spin down to collect the fluid

Adjust binding conditions

7. Add **1.000 µl Ethanol** (96-100 %) and mix thoroughly
8. Spin down to collect the fluid
9. Condition the column with 500 µl Buffer CC (use vacuum to run the buffer through the column)

Bind DNA to column matrix

10. Load the lysate to the funnel and apply vacuum until the lysate flowed through the column

Wash silica membrane

11. Add 1.000 µl wash buffer VW1 (apply vacuum to achieve flow through)
12. Add 500 µl wash buffer WB (apply vacuum to achieve flow through)

Dry silica membrane

13. Remove NucleoSnap column from the vacuum manifold insert it into a collection tube and disassemble funnel and column, discard the funnel
14. Centrifuge the column 13.000 x g, 3 min to remove residual ethanol

Elute cfDNA

15. Transfer the spin column to an elution tube
16. Add 50 µl elution buffer onto the centre of the membrane
17. Centrifuge 11.000 x g, 1 min
18. Discard the column and store the eluted cfDNA at -20°C or below until further analysis

## Publication bibliography

Breitbach, Sarah; Tug, Suzan; Helmig, Susanne; Zahn, Daniela; Kubiak, Thomas; Michal, Matthias *et al.* (2014): Direct quantification of cell-free, circulating DNA from unpurified plasma. In *PloS one* 9 (3), e87838. DOI: 10.1371/journal.pone.0087838.

Devonshire, Alison S.; Whale, Alexandra S.; Gutteridge, Alice; Jones, Gerwyn; Cowen, Simon; Foy, Carole A.; Huggett, Jim F. (2014): Towards standardisation of cell-free DNA measurement in plasma. Controls for extraction efficiency, fragment size bias and quantification. In *Analytical and bioanalytical chemistry* 406 (26), pp. 6499–6512. DOI: 10.1007/s00216-014-7835-3.

Schrage, Kathrin; Linden, Justus; Kämper, Martin; Reiter, Jenny; Schuster, Rainer (2022): Comparison of S-Monovette® cfDNA Exact with two blood collection tubes for stabilization of cfDNA. SARSTEDT AG & Co. KG. Nümbrecht. Available online at https://www.sarstedt.com/fileadmin/user_upload/Mediacenter/Studien/Studie_neu/an_009_comparison_of_smonovette_cfdna_exact_0922.pdf.

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